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Review on Lipophilic and Hydrophilic Extractives in Tissues of Common Beech

Pregled lipofilnih i hidrofilnih ekstraktivnih tvari u tkivima obične bukve

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ABSTRACT • *Common beech (Fagus sylvatica L.) is one of the most widespread and economically important tree species in Europe and, therefore, represents a potential source of high value added extractives. The aim of this paper was, therefore, to review the existing data regarding the composition of lipophilic and hydrophilic extractives of beech tissues, and the extraction systems and analytical techniques used for their examination. The lipophilic extractable fraction of beech is characterized mainly by saturated and unsaturated fatty acids, fatty alcohols and sterols while the hydrophilic extractives of beech consist of soluble sugars, i.e. monosaccharides, oligosaccharides, sugar alcohols and sugar acids, as well as of simple phenols and flavonoids. Chromatography has been recognized as the convenient and most frequently used technique for the chemical analysis of extractives. This overview showed that the information about the composition of low-molecular extractives of beech is satisfactory, but the data on oligomeric extractives are still fragmentary.*

Key words: *beech, extractives, fatty acids, sterols, sugars, phenols, catechin, chromatography*

SAŽETAK • *Obična bukva (Fagus sylvatica L.) jedna je od najrasprostranjenijih i gospodarski najvažnijih vrsta drva u Europi i stoga je potencijalni izvor ekstraktivnih tvari visoke dodatne vrijednosti. Cilj rada bio je preispitati postojeće podatke koji se odnose na sastav lipofilnih i hidrofilnih ekstraktivnih tvari u tkivima bukve te sustave za ekstrakciju i analitičke tehnike koje se primjenjuju za njihovo istraživanje. Lipofilne frakcije koje se mogu ekstrahirati iz bukve uglavnom su zasićene i nezasićene masne kiseline, masni alkoholi i sterol, dok se hidrofilne ekstraktivne tvari iz bukve sastoje od topljivih šećera, tj. monosaharida, oligosaharida, šećernih alkohola i šećernih kiselina, kao i od jednostavnih fenola i flavonoida. Kromatografija je prepoznata kao prikladna i najčešće primjenjivana tehnika kemijske analize ekstraktivnih tvari. Ovaj je pregled pokazao da su podaci o sastavu niskomolekularnih ekstraktivnih tvari iz bukve zadovoljavajući, ali su podaci o oligomernim ekstraktivnim tvarima još uvijek fragmentarni.*

Ključne riječi: *bukva, ekstraktivne tvari, masne kiseline, steroli, šećeri, fenoli, katehin, kromatografija*

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1 INTRODUCTION
1. UVOD

Utilization of hardwood represents one of the main industrial and research issues of the wood sector in Europe. Common beech (*Fagus sylvatica* L.) accounts for approximately one third of the wood stock in Slovenian forests (Report of Slovenian Forest Service for 2011) and represents an economically important tree species with a relatively high potential in wood industry. One of the main deficiencies of this tree species is its tendency to develop discoloured wood in the central part of the tree, also known as the red heart (Bosshard, 1974; Torelli, 1984). Red heart in beech has been investigated from various aspects, ranging from physiology, gross and minute anatomy, chemical composition and physical, mechanical and technological properties (Dietrichs, 1964a; Bauch, 1984; Sachsse, 1991; Torelli *et al.*, 1994; Baum and Schwarze, 2002; Koch *et al.*, 2003; Hofmann *et al.*, 2004; Wernsdörfer *et al.*, 2005; Oven *et al.*, 2008; Mali *et al.*, 2009; Vek *et al.*, 2014; Vek *et al.*, 2015). Generally, if compared to the unaffected wood, the value of discoloured round wood, as well as wood elements on the market, is usually lower (Zell *et al.*, 2004) and, therefore, attempts were made to commercially promote discoloured beech wood (Koch, 2002). In addition to the main structural components of the cell wall, i.e. cellulose, hemicelluloses and lignin, different tissues of a living tree also contain smaller amounts of compounds, which can be removed from the plant tissue with the process of extraction and are, therefore, known as extractives. These are natural low-molecular compounds, the non-structural components of wood, located in the lumina of cells and extraneous to a lignocellulose cell wall. From the physiological aspect of view, the extractive compounds of plant tissue are primary and secondary metabolites (Rowe and Conner, 1979; Fengel and Wegener, 1989; Kai, 1991; Holmbom, 1999). Primary metabolites are present in every plant species, whereas specific secondary metabolites can be found only in some tree species or related group of species. Secondary metabolites were sometimes referred to as accessory compounds (Fengel and Wegener, 1989), because their important ecological functions in the tree have not been understood for a long time. It is known that these in general contribute to protection of plants and against herbivories and microbial pathogens, serve as attractants for pollinators and seed-dispersing animals and function as agents of plant–plant competition and plant-microbe symbioses (Taiz and Zeiger, 2002).

Extractives can be classified according to their chemical similarities, with respect to biochemical paths of their synthesis or regarding the solvent, in which they are soluble. Based on solubility, extractives are divided into classes of lipophilic and hydrophilic extractives (Willför *et al.*, 2006; Jansson and Nilvebrant, 2009).

As proposed by Holmbom (1999), extractives are usually analyzed at three levels, i.e. gravimetric determination of total extractives, determination of different component groups and analysis of individual components. The first level of extractive analysis includes gravimetric and other determination of total extractives. It is generally known that the xylem of tree species in the temperate climate zone contains a relatively small amount of extractives, up to 5 – 10 % (Umezawa, 2000). Anyway, the concentration can be much higher in certain parts of the tree, e.g. in branch bases, bark and roots. Moreover, higher amounts of extractives are also found in some tropical and subtropical woods (Fengel and Wegener, 1989; Holmbom, 1999). As reported by Wagenführ (1996), beech wood consists of 33.7 % to 46.4 % of cellulose, 11.6 % to 22.7 % of lignin, 11.8 % to 25.5 % of hemicelluloses, 3 % to 5 % of extractives, meanwhile the share of inorganic compounds represents 0.3 % to 1.2 %. By applying standardized analytical methods used in wood chemistry, 47.66 % of cellulose, 25.53 % of lignin, 69.01 % of holocellulose, 0.93 % substances soluble in benzene-alcohol, 2 % of substances soluble in hot water, 13.15 % substances soluble in 1 % NaOH and 0.3 % of mineral substances (ash) were determined by Bodirlau *et al.* (2008). The pH value of beech wood ranges from 5.06 up to 5.13 (Hillis, 1987). Fengel and Wegener (1989) stated that the average pH value is 5.4. Even more, authors reported that the pH value of cold water extract is 5.5, while the pH value of hot water extract is slightly lower (5.3). Sixta *et al.* (2004) found 24.2 % of lignin in freshly prepared beech wood chips. Further carbohydrate analysis revealed 41.6 % glucan, 17.9 % xylan, 1.3 % mannan, 1.2 % galactan and 0.5 % arabinan (Sixta *et al.*, 2004). It was reported that intact sapwood contains less lignin (19.75 %) than biologically decayed wood (20.82 %) (Košikova *et al.*, 2008), whereas the origin of decay was not described in this research. Generally, beech wood is characterized by a relatively low amount of extractives (Rowe and Conner, 1979). Furthermore, the information regarding the content and composition of extractives in beech wood is poor. In the case of fresh beech wood chips, Sixta *et al.* (2004) stated that 0.2 % of the compound are extracted with dichloromethane, 1.0 % with acetone, 1.7 % of extractives are soluble in ethanol, while 2.7 % are obtained with water. According to Košikova *et al.* (2008), in comparison to healthy sapwood, from which 1.78 % of extractives were gained, decayed wood contains less extractives soluble in acetone, i.e. 0.98 %. Furthermore, Kubel and Weissmann (1988) report that the amount of extractives, soluble in petrol ether and diethyl ether, is relatively low and it amounts to 0.2% and 0.1%, respectively. More compounds can be extracted by the mixture of alcohols and water, 1.6 % with acetone/water (9:1, v/v) and 1.2 % with ethanol/water (8:2, v/v). A relatively low amount of water soluble extractives (0.3 %) can be attributed to the successive extraction procedure.

The basic gravimetrical estimation of the amount of total extractives is usually upgraded by the second and third level of analyses, i.e. determination of the component groups in extracts and evaluation of composition of individual extractives. For the determination of groups of extractives in wood extracts, e.g. total phenols, flavonoids or proanthocyanidins, a spectrophoto-

metric analysis (UV-Vis) represents a quick and reliable technique (Baum and Schwarze, 2002; Albert *et al.*, 2003; Brighente *et al.*, 2007; Vek *et al.*, 2013a; Vek *et al.*, 2013b). The lack of semi-qualitative evaluation is that UV-Vis analysis gives relative and not absolute results as a consequence that the amount of component group is estimated towards the component of external standard, e.g. gallic acid, quercetin of catechin. Therefore, more precise chromatographic techniques are used, i.e. thin-layer chromatography (TLC), gas chromatography (GC) or high performance liquid chromatography (HPLC). Qualitative determination is possible by the ^{13}C NMR and eventually with Furrier transform infrared spectroscopy (FTIR) as well. Furthermore, for the determination of different groups of extractives by means of HPLC, both the size-exclusion (HPLC-SEC) and reversed-phase (RP) modes can be applied. Precise qualitative evaluation of individual compounds separated by chromatographic techniques (GC or HPLC) is most conveniently made by mass spectrometry (MS). Therefore, GC/MS and HPLC/MS represent an adequate technique for the identification of extractives in complex mixtures such as plant extract.

The aim of the present work was to review the literature on extractives, which have been qualitatively evaluated as the extracts of beech tissue so far. In the following, the results of various chemical analyses of extractives occurring in different types of beech tissue are presented separately for lipophilic and hydrophilic components (Holmbom, 1999; Naczk and Shahidi, 2007).

2 EXTRACTIVES OF BEECH
2. EKSTRAKTIVNE TVARI U TKIVIMA BUKVE

2.1 Lipophilic extractives
2.1. Lipofilne ekstraktivne tvari

Lipophilic extractives are referred to as the compounds which are soluble in nonpolar organic solvents, e.g. pentane, hexane, petroleum ether, dichloromethane, chloroform or toluene. It is well known that lipophilic extractives can have a negative influence and may disturb the analysis of more polar compounds. Moreover, they may have a deleterious effects on chromatographic instrumentation due to column clogging (Slanina and Glatz, 2004). Therefore, it is recommended first to remove the lipophilic extractives from the sample before further extraction with polar solvents (Naczk and Shahidi, 2004; Willför *et al.*, 2006). Waxes, fats, fatty alcohols, fatty acids, terpenoids, sterols and steryl esters are known as the characteristic representatives of lipophilic extractives. Some of them were also identified in the nonpolar extractible fraction of beech.

In the case of beech wood dust, extracts were characterized by a relatively low amount of lipophilic

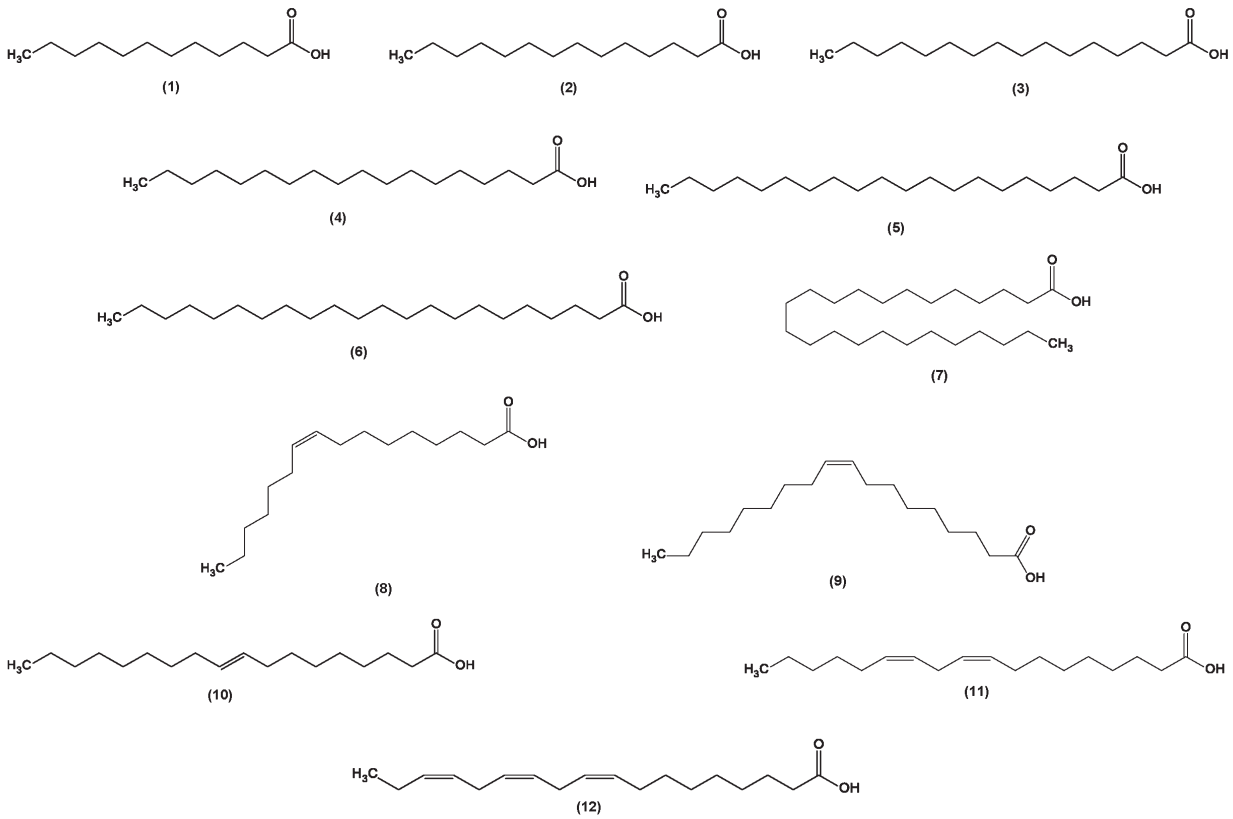

Figure 1 Structure formulas of saturated and unsaturated fatty acids identified in the extracts of beech: (1) Lauric C12:0 acid; (2) Myristic C14:0 acid; (3) Palmitic C16:0 acid; (4) Stearic C18:0 acid; (5) Arachidic C20:0 acid; (6) Behenic C22:0 acid; (7) Lignoceric C24:0 acid; (8) Palmitoleic C16:1 acid; (9) Oleic C18:1 acid; (10) Elaidic C18:1 acid; (11) Linoleic C18:2 acid; (12) Linolenic C18:3 acid

Slika 1. Strukturne formule zasićenih i nezasićenih masnih kiselina identificiranih u ekstraktivnim tvarima bukve: (1) laurinska C12:0 kiselina; (2) miristinska C14:0 kiselina; (3) palmitinska C16:0 kiselina; (4) stearinska C18:0 kiselina; (5) arahidonska C20:0 kiselina; (6) behenska C22:0 kiselina; (7) lignocerinska C24:0 kiselina; (8) palmitoleinska C16:1 kiselina; (9) oleinska C18:1 kiselina; (10) elaidinska C18:1 kiselina; (11) linolna C18:2 kiselina; (12) linolenska C18:3 kiselina

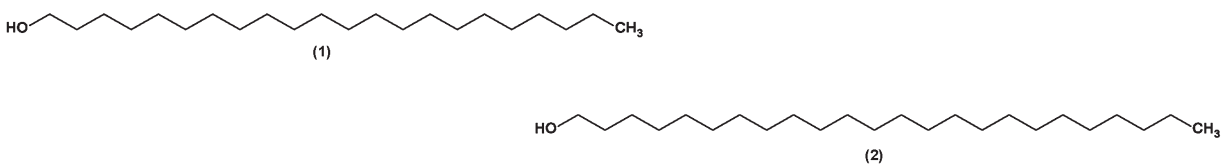

Figure 2 Structures of fatty alcohols found in the cyclohexane extracts of beech wood: (1) Behenyl C22:0 alcohol; (2) Lignoceryl C24:0 alcohol
Slika 2. Strukture masnih alkohola pronađenih u cikloheksanskim ekstraktima bukovine: (1) behenil C22:0 alkohol; (2) lignocerinski C24:0 alkohol

extractives (Kubel and Weissmann, 1988). In the non-polar extracts of sapwood and wood chips of beech, saturated and unsaturated fatty acids were identified as the palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acid by means of different chromatographic techniques (Kubel and Weissmann, 1988; Zule and Može, 2003). Moreover, a saturated lauric (C12:0), myristic (C14:0), arachidic (C20:0), behenic (C22:0) and lignoceric acid (C24:0) and unsaturated palmitoleic (C16:1), elaidic (C18:1), linolenic (18:3), dehydroabietic and unsaturated hydroxyoctadecadiene acid were identified in the extracts of sapwood as well (Figure 1 Structure formulas of saturated and unsaturated fatty acids identified in the extracts of beech: (1) Lauric C12:0 acid; (2) Myristic C14:0 acid; (3) Palmitic C16:0 acid; (4) Stearic C18:0 acid; (5) Arachidic C20:0 acid; (6) Behenic C22:0 acid; (7) Lignoceric C24:0 acid; (8) Palmitoleic C16:1 acid; (9) Oleic C18:1 acid; (10) Elaidic C18:1 acid; (11) Linoleic C18:2 acid; (12) Linolenic C18:3 acid (Figure 1) also represent a part of the aliphatic fraction in suberin (Perra *et al.*, 1993), a hydrophobic biopolymer, whose occurrence is characteristic for wound response of wood in beech (Pearce, 1996; Torelli and Oven, 1996; Oven *et al.*, 1999; Pearce, 2000; Schwarze and Baum, 2000). The presence of fatty alcohols, i.e. behenyl C22:00 alcohol and lingoceryl C24:0 (Figure 2 Structures of fatty alcohols found in the cyclohexane extracts of beech wood: (1) Behenyl C22:0 alcohol; (2) Lignoceryl C24:0 (Figure 2), has been recently proven in the cyclohexane extracts of wood of red hearted beech by the long-column GC/MS analysis (Vek *et al.*, 2014). Besides fatty acids, the extracts of beech knots are also characterized by a low concentration of resin acids (Lindberg *et al.*, 2004).

In addition to fatty acids and alcohols, numerous glycerides, sterols and steryl esters were identified as the lipophilic extractives of beech wood. An acyclic triterpenoid squalene (Figure 3) and cyclic cycloartenyl acetat, β-amyrin acetate, acetyl methyl betulinate and dihydrositosterol were extracted from dried wood by Pišova and Souček (1973). Furthermore, relatively low levels of β-carotene and lutein were found in the

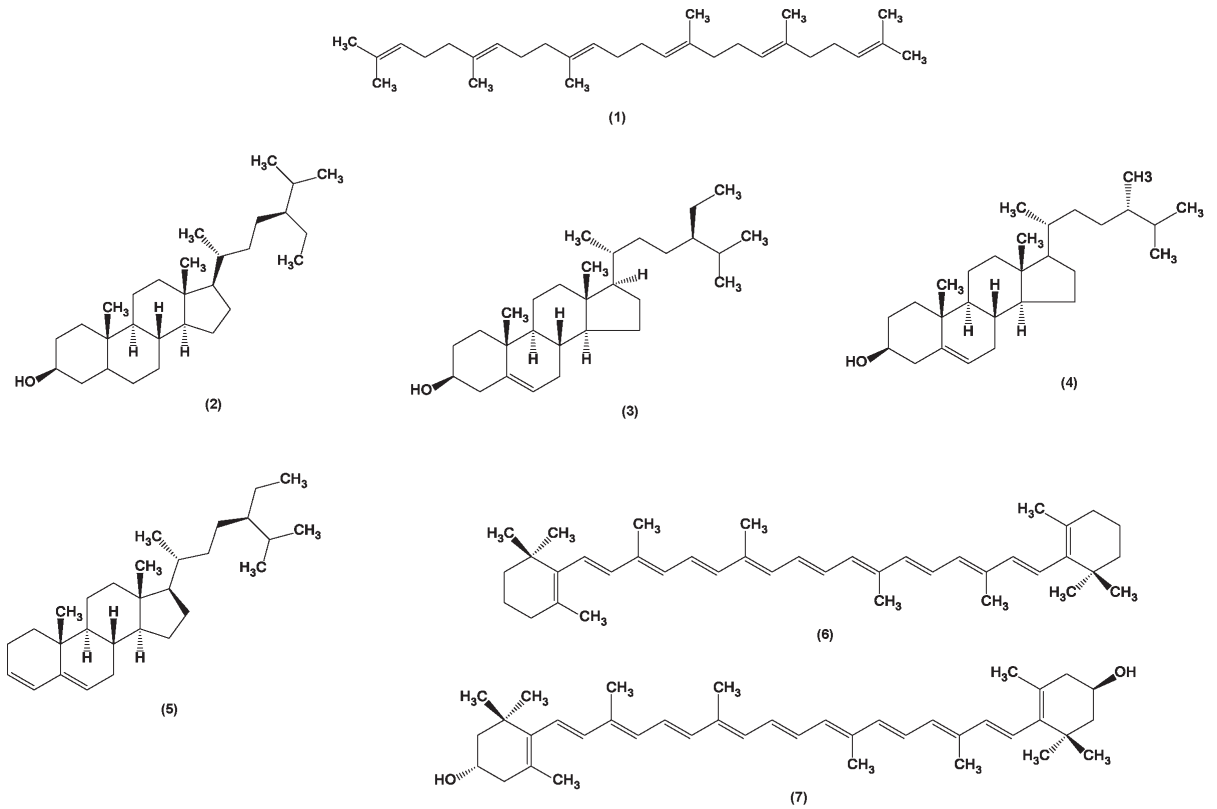

Figure 3 Structure formulas of terpenoids, i.e. acyclic triterpene, sterols tetraterpenes, occurring in beech wood: (1) Squalene; (2) β-sitostanol; (3) β-sitostanol; (4) Campesterol; (5) Stigmastadiene; (6) β-carotene; (7) Lutein.
Slika 3. Strukturne formule terpenoida, tj. acikličkih triterpena, sterolnih tetraterpena koji se pojavljuju u drvu bukve: (1) skvalen; (2) β-sitostanol; (3) β-sitostanol; (4) kampesterol; (5) stigmastadien; (6) β-karoten; (7) lutein

sapwood extracts of beech by Masson *et al.* (1997). This was actually the first report about the presence of carotenoids in wood tissue (Figure 3). As shown in Figure 3, a β-sitosterol, dihydrositosterol (β-sitostanol) and campesterol were qualitatively evaluated as the free sterols occurring in various beech materials, i.e. sapwood dust, wood chips and dried wood (Pišova and Souček, 1973; Kubel and Weissmann, 1988; Zule and Može, 2003). Furthermore, Zule and Može (2003) described β-sitosterol as the most important unesterified sterol in beech wood with a very significant physiological function in wood. Sterols were also found in beech knots (Lindberg *et al.*, 2004). As recently reported (Vek *et al.*, 2014), a characteristic compound of the nonpolar fraction of extractives in beech wood is also stigmasta-3,5-diene (Figure 3) that belongs to the group of sterenes, known as the dehydration compounds of sterols (Gallina Toschi *et al.*, 1996; Amelio *et al.*, 1998). Lindberg *et al.* (2004) reported on the presence of sterols in the extract of beech knots.

2.2 Hydrophilic extractives
2.2. Hidrofilne ekstraktivne tvari

After removing lipophilic extractives, the hydrophilic compounds are usually further extracted by means of more polar solvents, such as methanol, acetone, ethanol, water, etc. Soluble sugars and various phenolic extractives, e.g. simple phenolics, stilbenes, lignans, flavonoids and tannins are known as the characteristic representatives of hydrophilic extractives. In a broader sense, inorganic compounds are also known as hydrophilic extractives and they can be extracted by water (Fengel and Wegener, 1989). The yield of hydrophilic extractives from beech wood is usually much larger than that of lipophilic extractives (Kubel and Weissmann, 1988).

Various monosaccharides and oligosaccharides, sugar alcohols and acids, simple phenolics, flavonoids, both oligomeric units of tannins have been determined as the hydrophilic substances of beech wood. Among the monosaccharides, glucose, galactose, arabinose, fructose, xylose, mannose and rhamnose were identified in the wood extracts and wood condensate of beech (Dietrichs, 1964b; Kubel and Weissmann, 1988; Irmouli *et al.*, 2002). Furthermore, disaccharides saccharose and trehalose as well as trisaccharide raffinose were identified in the extracts of beech wood (Dietrichs, 1964b; Vek *et al.*, 2014). The presence of both tetrasaccharide stachyose and polysaccharide starch was reported for the sapwood and wood condensate of beech, respectively (Dietrichs, 1964b; Irmouli *et al.*, 2002). Sugar alcohols, i.e. erytritol, arabitol, sorbitol, mannitol, xylitol

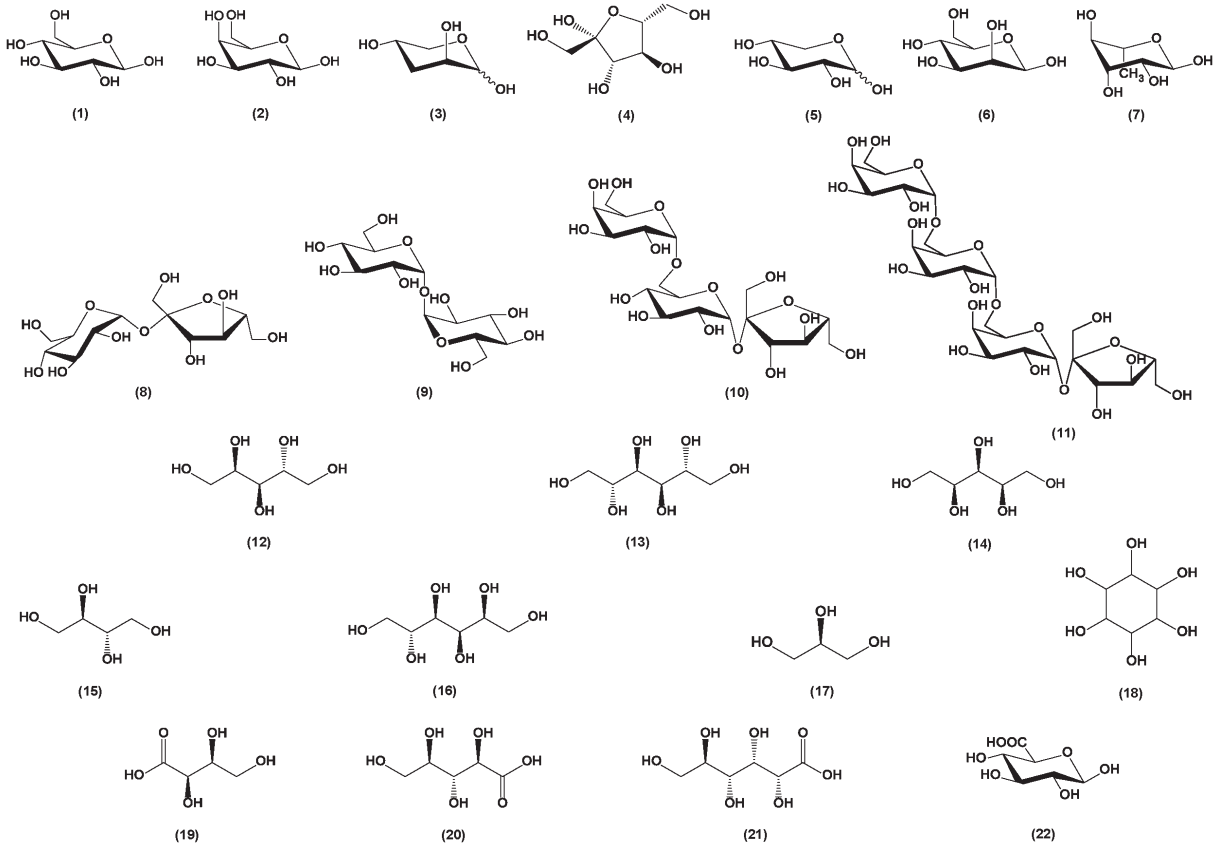

Figure 4 Chemical structures of monosaccharides, oligosaccharides, sugar alcohols and sugar acids identified in beech wood: (1) Glucose; (2) Galactose; (3) Arabinose; (4) Fructose; (5) Xylose; (6) Mannose; (7) Rhamnose; (8) Saccharose; (9) Trehalose; (10) Raffinose; (11) Stachyose; (12) Arabitol; (13) Mannitol; (14) Xylitol; (15) Erytritol; (16) Sorbitol; (17) Glicerol; (18) Inositol; (19) Threonic acid; (20) Ribonic acid; (21) Gluconic acid; (22) Glucuronic acid
Slika 4. Kemijska struktura monosaharida, oligosaharida, šećernih alkohola i šećernih kiselina identificiranih u drvu bukve: (1) glukoza; (2) galaktoza; (3) arabinoza; (4) fruktoza; (5) ksiloza; (6) manoza; (7) ramnoza; (8) saharoza; (9) trehaloza; (10) rafinoza; (11) stahioza; (12) arabitol; (13) manitol; (14) ksilitol; (15) eritritol; (16) sorbitol; (17) glicerol; (18) inozitol; (19) treonska kiselina; (20) ribonska kiselina; (21) glukonska kiseline; (22) glukuronska kiselina

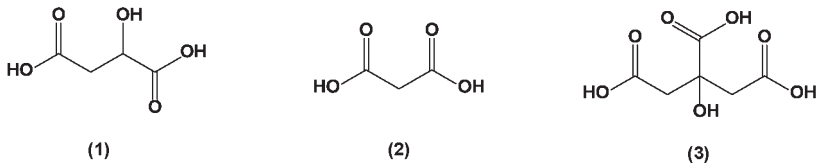

Figure 5 Structure formulas of carboxylic acids identified in methanolic extracts of beech wood: (1) Malic acid; (2) Malonic acid; (3) Citric acid
Slika 5. Strukturne formule karboksilnih kiselina identificiranih u metanolnim ekstraktima iz bukovine: (1) jabučna kiselina; (2) malonska kiselina; (3) limunska kiselina

and glycerol, as well as inositols were qualitatively evaluated in extracts of wound-associated tissues and knots of beech (Zule and Može, 2003; Vek *et al.*, 2014). In addition to sugar alcohols, the occurrence of several sugar acids (ribonic, threonic, gluconic and glucuronic acid) has been proven for the methanol extracts of stem wood by means of GC/MS (Figure 4). Our recent chromatographic study has also shown the presence of low-molecular carboxylic acids in the extracts of both stem wood and knots of beech, i.e. malic, malonic and citric acid (Figure 5) (Vek *et al.*, 2014).

Numerous simple phenols have been extracted from beech wood as well. Challinor (1996) applied gas chromatography coupled with mass spectrometry for the qualitative evaluation of extractives in the wood shavings/turnings of intact beech. As the characteristic compounds in the tetramethylammonium hydroxide extracts, 5-hydroxy, 2-hydroxymethyl pyranone (triv. kojic acid), 3,4-dimethoxybenzaldehyde, 3,4-dimethoxybenzoic acid methyl ester, 3,4,5-trimethoxybenzaldehyde and 3,4,5-trimethoxybenzoic acid methyl ester were found. From thermal treated wood, i.e. steamed and dried beech wood, a sinapyl alcohol, coniferyl alcohol, 2,6-dimethoxybenzoquinone, protocatechuic acid, vanillic acid, vanillin, syringic acid, coniferaldehyde, siringalydehide synapic acid, gallic acid, p-hy-

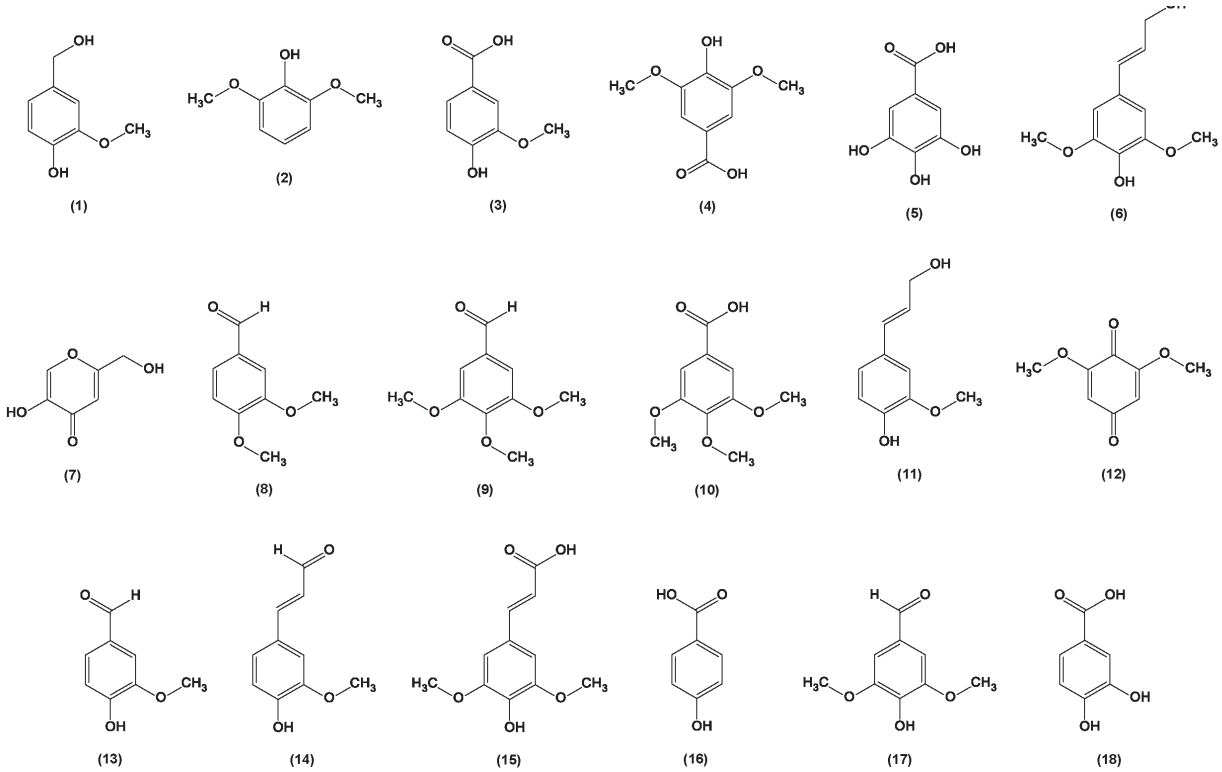

Figure 6 Structure formulas of simple phenolics identified in beech wood extracts: (1) Vanilyl alcohol; (2) Syringol; (3) Vanillic acid; (4) Syringic acid; (5) Gallic acid; (6) Sinapyl alcohol; (7) Kojic acid; (8) 3,4-Dimethoxybenzaldehyde; (9) 3,4,5-Trimethoxybenzaldehyde; (10) 3,4,5-trimethoxybenzoic acid; (11) Coniferyl alcohol; (12) 2,6-Dimethoxybenzoquinone; (13) Vanilin; (14) Coniferaldehyde; (15) Synapic acid; (16) p-Hydroxybenzoic acid; (17) Syringaldehyde; (18) Protocatechuic acid
Slika 6. Strukturne formule jednostavnih fenola identificiranih u ekstraktima iz bukovine: (1) vanilil alkohol; (2) siringol; (3) vanilinska kiselina; (4) siringijska kiselina; (5) galna kiselina; (6) sinapil alkohol; (7) Kojić kiselina; (8) 3,4-dimetoksibenzaldehid; (9) 3,4,5-trimetoksibenzaldehid; (10) 3,4,5-trimetoksibenzojeva kiselina; (11) koniferil alkohol; (12) 2,6-dimetoksibenzokinon; (13) vanilin; (14) koniferaldehid; (15) sinapik kiselina; (16) p-hidroksibenzojeva kiselina; (17) siringaldehid; (18) protokatehična kiselina

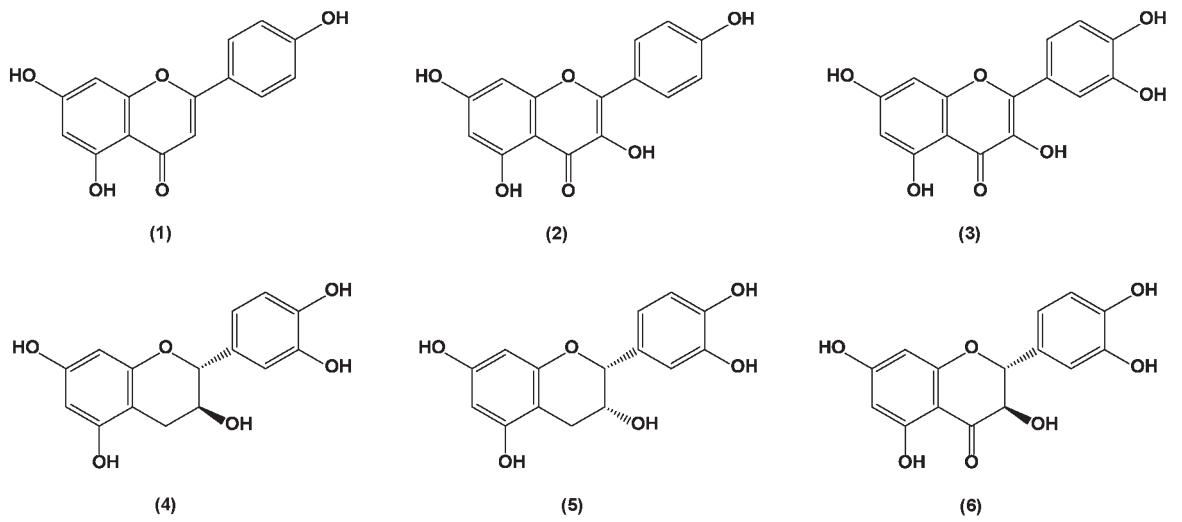

Figure 7 Flavonoids found in the extracts of functional wood, discoloured wood, dried wood, leaves, bark and roots of beech: (1) Apigenin; (2) Kaempferol; (3) Quercetin; (4) Catechin; (5) Epicatechin; (6) Taxifolin
Slika 7. Flavonoidi pronađeni u ekstraktima funkcionalnog drva, izbijeljenog drva, osušenog drva, lišća, kore i korijena bukve: (1) apigenin; (2) kempferol; (3) kvercetin; (4) katehin; (5) epikatehin; (6) taksifolin

droxybenzoic acid were successfully extracted and qualitatively evaluated (Irmouli *et al.*, 2002; Koch *et al.*, 2003; Mounguengui *et al.*, 2007; Vek *et al.*, 2014). Moreover, vanillin, vanillic acid and syringic acid are also known as the constituents of phenolic fraction of a depolymerized suberin in beech (Perra *et al.*, 1993). In addition to simple phenols, the presence of aromatic dimeric structures, i.e. lignin-type compounds, was also proven in beech wood extracts (Koch *et al.*, 2003; Vek *et al.*, 2014). These dimers made up of coniferyl and sinapyl alcohol were referred to as the precursors of a lignin biosynthesis accordingly to Koch *et al.* (2003), but they can represent another group of phenolic extractives, called lignans. Lignans were found in extracts of beech knot by Lindberg *et al.* (2004). Furthermore, they reported about the presence of small amounts of stilbenes, generally known as bioactive compounds with the 1,2-diphenylethene structure (Lindberg *et al.*, 2004). Some of simple phenolic compounds that have been shown as the extractives of beech wood are presented in Figure 6.

Flavonoids are commonly referred to as both the most important and abundant group of extractives of beech tissue (Brignolas *et al.*, 1995; Pearce, 1996; Torelli, 2003; Vek, 2013). Catechin is the most frequently reported flavonoid that can be extracted from the beech wood (Figure 7). A few research groups reported on the presence of catechin in the sapwood and discoloured wood (Kubel and Weissmann, 1988; Baum and Schwarze, 2002; Koch *et al.*, 2003; Zule and Može, 2003; Hofmann *et al.*, 2004; 2008). Catechin was also found in the extracts of thermally treated and decayed beech wood (Mounguengui *et al.*, 2007; Lekounougou *et al.*, 2008). It was reported that the amount of catechin in discoloured tissues decreases due to its participation in the process of formation of colour chromophores (Hofmann *et al.*, 2004). It was also suggested that, during thermal treatment of beech wood, the condensation of catechin contributes to a brown stain pigment, whereas other extractives, e.g. a 2,6-dimethoxybenzoquinone (Figure 6) and taxifolin (Figure 7), contribute to the final colour of discoloured tissues (Koch *et al.*, 2003). Therefore, the discolouration mechanism in beech wood can be understood as a condensation of catechin monomers to polymer forms. Besides wood, the presence of flavonoids has also been shown for the extracts of other parts of beech tree, e.g. leaves, knots, roots and bark (Beyeler and Heyser, 1997; Dubeler *et al.*, 1997; Feucht *et al.*, 1997; Lindberg *et al.*, 2004; Pietarinen *et al.*, 2006; Pirvu *et al.*, 2010). In addition to the catechin, its diastereomer epicatechin was also found both in beech wood and in roots and leaves (Beyeler and Heyser, 1997; Feucht *et al.*, 1997; Hofmann *et al.*, 2004). As reported by Pirvu *et al.* (2010), a flavone apigenin, flavonols kaempferol and quercetin and various caffeic acid derivates can be extracted with ethanol from beech leaves (Figure 7). Recently, a comprehensive investigation, with the high-performance liquid chromatography and multistage mass spectrometry, has shown the presence of catechin and epicatechin in beech bark extracts (Hofmann *et al.*, 2015).

Phenolic compounds frequently occur as glycosides as the core aglycone in plant tissues. According to Sherwood and Bonello (2013), the sugar unit of phenolic glycoside serves to improve solubility for storage in cell organs, e.g. vacuoles. Some research groups described the presence of flavonoid glycosides as characteristic for beech extracts (Figure 8). Catechin glucoside and taxifolin glucoside were found in the extracts of both normal and thermally treated beech wood (Kubel and Weissmann, 1988; Koch *et al.*, 2003). Numerous glycosides, i.e. taxifolin-*O*-pentoside, taxifolin-*O*-hexoside, quercetin-*O*-pentoside and quercetin-*O*-hexoside were proven to occur in the methanol extracts of wood dust by Mammela (2001). The presence of glycosides was confirmed in the extracts of beech bark as well. Thus, *cis*-coniferin, *cis*-syringin,

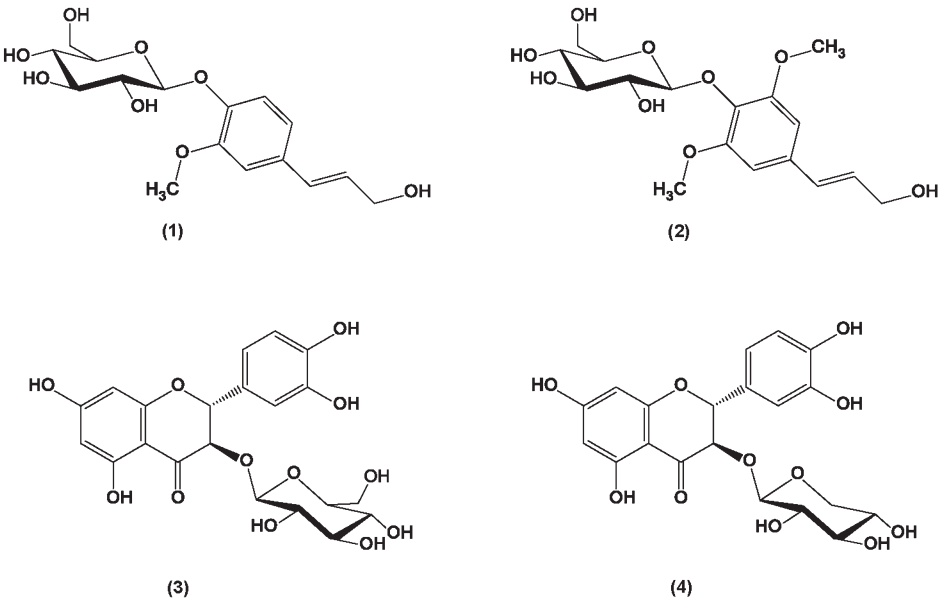

Figure 8 Chemical structures of determined flavonoid glycosides occurring in beech tissues: (1) Coniferin; (2) Syringin; (3) Taxifolin-3-glucopyranoside; (4) Taxifolin-3-xylopyranoside
Slika 8. Kemijska struktura utvrđenih flavonoida glikozida koji se pojavljuju u tkivima bukve: (1) koniferin; (2) siringin; (3) taksifolin-3-glukopiranozid; (4) taksifolin-3-ksilopiranozid

cis-isoconiferin, (2*R*,3*R*)-(+)-glucodistylin, (2*S*,3*S*)-(–)-glucodistylin and 2*R*,3*R*-taxifolin-3-*D*-*β*-xylopyranosid were identified and completely assigned by 2D NMR techniques (Dubeler *et al.*, 1997). More isomers of taxifolin-O-hexosides as well as taxifolin-O-pentosides have been qualitatively and quantitatively evaluated in beech bark by means of HPLC-MS/MS analysis (Hofmann *et al.*, 2015). Authors report about the presence of syringin, coniferins, quercetin-O-hexoside, coumaric acid di-O-hexoside, syringic acid-di-O-hexosides, coniferyl alcohol-O-hexoside-O-pentoside in beech bark extracts as well (Hofmann *et al.*, 2015).

Flavonoids can also occur in wood extracts as the aglicon polymers (Fengel and Wegener, 1989). The latter are known as the condensed tannins or proanthocyanidins. It has to be mentioned that the term proanthocyanidins describes bioflavonoids, leucoantocyanidins as well as condensed tannins. The building blocks of most proanthocyanidins are the flavanols catechin and epicatechin (Dixon et al., 2005), i.e. the characteristic compounds of flavonoid fraction of beech wood extracts. Regarding the inter flavanol linkages as well as the degree of polymerization, the proanthocyanidins are generally divided into A-, B- or C-type proanthocyanidins (Figure 9). Proanthocyanidins were spectrophotometrically evaluated in extracts of intact sapwood as well as in reaction zone of beech by Scalbert *et al.* (1989) and Baum and Schwarze (2002). This semi-quantitative technique has also been applied for the determination of proanthocyanidins in the stem and knot samples of red hearted beech (Vek *et al.*, 2014). Koch *et al.* (2003) also reported about the presence of oligomeric flavonoids in acetone-water and methanol-water extracts of beech wood. A relatively large amount of oligomeric polyphenols is characteristic for beech knotwood (Lindberg *et al.*, 2004; Vek *et al.*, 2014). Just recently, Hofmann *et al.* (2015) have identified and quantified a large number of dimeric and trimeric procyanidins in beech bark by

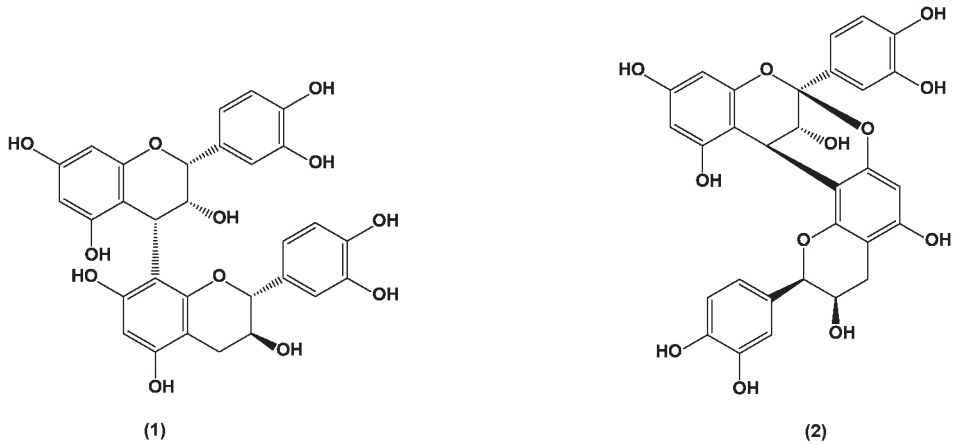

Figure 9 Chemical structures of simple dimeric units of type B1 (1) and type A2 (2) proanthocyanidins
Slika 9. Kemijska struktura jednostavnih dimernih jedinica tipa B1 (1) i tipa A2 (2) proantocianidina

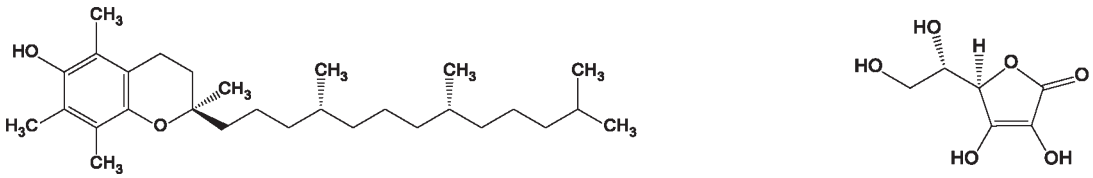

Figure 10 α-tocopherol (Vitamin E) (1) and Ascorbic acid (Vitamin C) (2) as identified in the extracts of beech for leaves and wood

Slika 10. (1) α-tokoferol (vitamin E) i (2) askorbinska kiselina (vitamin C), kao što je identificirano u ekstraktima bukve, u listovima i u drvu

HPLC-MS/MS. Further on, proanthocyanidins were also identified in the extracts of roots and leaves (Feucht *et al.*, 1994; Beyeler and Heyser, 1997; Feucht *et al.*, 1997). Condensed tannins, present in beech leaves, were investigated by Behrens *et al.* (2003). The authors demonstrated that proanthocyanidins of beech leaves are built up of procyanidin (catechin and epicatechin) and prodelphinidin units (gallocatechin and epigallocatechin).

Different antioxidants were identified in the beech leaf extracts, i.e. vitamin C (ascorbic acid) and vitamin E (α-tocopherol) (Kunert and Ederer, 1985). Structural formulas for both compounds are presented in Figure 10. It was shown that vitamin E also occurs in the tissues at the bases of living and dead breaches of beech. Furthermore, it can be extracted from these tissues by means of non-polar solvent, i.e. cyclohexane (Vek *et al.*, 2014). Further to the above, vitamin E could be classified as the lipophilic extractive as well.

After all, some of characteristic hydrophilic extractives occurring in tissues of beech trees are biologically active compounds, which make this forest biomass interesting for extraction and further utilization. For instance, antifungal properties against some basidiomycetes were found for catechin and taxifolin by Malterud *et al.* (1985). Some years later, epicatechin was also reported as the compound with antifungal activity (Ardi *et al.*, 1998; Baum and Schwarze, 2002). In addition to flavonoids, aglycones of *cis*-coniferin *and cis*-syringin, i.e. coniferyl alcohol and syringic acid, identified in the methanolic extracts of beech bark were reported as fungicides by Dubeler *et al.* (1997). Not only pure compounds, but also acetone extract of a beech knot, which was reported to be composed mainly of catechins and flavonoids, showed evident antibacterial properties (Välimaa *et al.*, 2007). Wood and bark extractives of various tree species have attracted much attention due to their possible application in various fields, including nutritional, cosmetic, medical and pharmaceutical industry, as natural biocides, feed additives or in industry of adhesives and leather production etc. Replacement of synthetic, artificial chemicals with more benign natural products, whose recovery has only little or no impact on human health and environment, is one of the important challenges in the field of biorefining forest biomass (Holmbom, 2011).

3 CONCLUSION
3. ZAKLJUČAK

It can be summarized that successive extraction using the non-polar and polar solvent, respectively, represents the most frequently used and appropriate way of extraction of different beech material. The extractives can be obtained from grinded samples by using magnetic stirrer, whereas, more aggressive extraction techniques are recommended, e.g. conventional Soxhlet apparatus or various subcritical systems for accelerated extraction (Thurbide and Hughes, 2000; Vek, 2013). From the analytical aspect of view, chromatography in combination with mass spectrometry has been found as the convenient technique for the qualitative and quantitative analysis of extractives. Since stem-wood of beech is generally characterized by the relatively low amounts of extractable compounds, there is a strong need to recognize the appropriate source for extraction. Knots of trees have been presented more times as a very rich source of different polyphenols, perhaps the richest in all of nature as reported by Holmbom (2011) and beech is not an exception (Vek, 2013). Furthermore, a very important step for recovering the target compounds from different parts of a beech tree is the optimization of appropriate and efficient extraction systems (i.e. extraction techniques and solvents) as it was recently demonstrated for beech bark (Hofmann et al. 2015). This short review revealed that the composition of low-molecular fraction of lipophilic and hydrophilic extractives is relatively well investigated, whereas the data on oligomeric fraction of extractable compounds are extremely sparse and require further research efforts.

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